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NUTRITION REVIEWS

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NUTRITION REVIEWS

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NUTRITION AND AGING

In contrast to the extensive and well controlled studies on nutrition and aging in the rat, initiated by McCay in 1929 and now in progress for over twenty years, the investigations on nutritional aspects of aging in human individuals are few and somewhat inhomogeneous. The demonstration by McCay and his associates, repeatedly confirmed, that caloric restriction during the growth period of rats results in a notable increase in the life span of this animal probably represents the most significant contribution so far made in the field of nutrition and aging. To what extent, if any, the observations made in the rat are applicable to human physiology remains uncertain, but the findings in the animal experiments raise the question whether the current emphasis on a high caloric intake by school children is well advised.

Although the procurement of data in human individuals along the lines employed in McCay's experiments would seem impossible, it must nevertheless be pointed out from a gerontologic side that the demonstration of a highly significant effect of caloric restriction during youth on the length of life in rats represents the only procedure which so far has been shown experimentally to be capable of prolonging life to a notable extent. It might perhaps be suggested that an approach to this problem in humans could be made by actuarial observations on individuals who for a period of several years during their childhood were involuntarily subjected to a restricted caloric intake, as was for instance the case in central European countries during and after the first World War. Even though the diet during this period was deficient in specific nutrients as well as in total calories and the conditions therefore not directly comparable with those prevailing in McCay's experiments, the importance of this problem in the field of gerontology would seem to warrant a statistical follow-up investigation, and such a study might well be undertaken by the World Health Organization of the United Nations.

Although the volume of research work in the field of nutrition of old individuals has shown some increase during the last five years (*see, e.g.*, J. E. Kirk, *J. Gerontol.* **6**, *70 (1951)*), the time still seems far away when it may be possible to establish recommended dietary standards for this age group. A major obstacle for the progress of research stems from the fact that the nutritional studies forced by circumstances chiefly are limited to institutionalized old individuals. It must be admitted that such studies may yield valuable information regarding specific nutritional problems, but the much higher incidence of debilitating disease among the inmates of infirmaries and hospitals as compared with noninstitutionalized old persons prevents the general extension of research data from the 3 per cent institutionalized to the 97 per cent noninstitutionalized old individuals. The introduction in recent years by Bessey, Lowry and their associates of analytic procedures for determination of various vitamins in capillary blood samples might, however, make possible a more extensive study of the nutritional condition of the active section of the old population, and the success already encountered with the use of such methods in the well-known Newfoundland survey entails some promise for the employment of a similar approach in the field of gerontology. The chances for acquiring the cooperation of normal elderly individuals for nutritional balance studies are probably much less favorable.

Since wasting of body tissues at the present time is believed to be one of the

most important and conspicuous changes in old age, a discussion of the relation of nutrition to this phenomenon would seem appropriate. It is generally contended that tissue wasting is conditioned by an insufficient protein synthesis by the body cells, but data have not been provided to show whether the protein synthesis in old age is actually decreased, or whether the wasting is caused by an increased catabolism of protein in the face of an unchanged rate of protein synthesis. It may be expected that an application of the technic with isotopic nitrogen employed by H. D. Hoberman in the rat (*Yale J. Biol. Med.* **22,** *341* (*1950*)) could yield information about this important problem. The possibility of muscular atrophy in old individuals being a simple sequence of inactivity must of course also be considered.

It has been suggested by F. Albright (*Ann. Int. Med.* **27,** *861* (*1947*)) that an increased tissue breakdown in old individuals is caused chiefly by the change occurring with age in the ratio between the catabolic corticosteroids and the anabolic androgens and estrogens. Investigations in the last few years have shown that the corticosteroid production by the adrenals remains practically unchanged from youth to old age, whereas a notable decrease in the androgen production by men and women and in the estrogen formation by women usually takes place with advancing years. These findings, based on increasingly reliable methodologic technics, at least confirm the premises for Albright's interesting theory. A more positive support for the hypothesis is provided by the observation that administration of testosterone in old individuals is capable of producing positive nitrogen balance and weight gain, apparently associated with some increase in voluntary muscle tissue.

The finding in old age of a relatively high incidence of negative nitrogen balance has been brought out in several publications (W. B. Kountz, L. Hofstatter, and P. Ackermann, *Geriatrics* **2,** *173* (*1947*); **3,** *171* (*1948*)). It seems important to the reviewer, however, to point out that such observations cannot necessarily be taken to imply that a primary metabolic disturbance is the cause of the negative balance. In many instances the negative balance has merely been conditioned by an insufficient total caloric or protein intake, which in young adults also would have been associated with a negative balance. In several other old patients, in whom a negative nitrogen balance has been registered, complicating diseases like chronic urinary or bronchial infections and mild cardiac insufficiency have been present, and such conditions are known to be capable of producing negative nitrogen balance irrespective of the age of the individual. Since the incidence of disease increases rapidly for each decade after the age of 60 the frequent finding of negative nitrogen balance in institutionalized old subjects is not in itself surprising. What would seem particularly needed are nitrogen balance studies in old individuals who are free from complicating diseases.

In a recent study by W. B. Kountz, L. Hofstatter, and P. G. Ackermann (*J. Gerontol.* **6,** *20* (*1951*)) on 4 elderly men, who were apparently free from complicating disease, the interesting finding was made that diets containing 0.5 g. of protein per kilogram of body weight were ineffective in keeping the individuals in nitrogen balance, whereas with an increase of the protein content to 0.7 g. equilibrium was obtained. Since it is usually possible to maintain nitrogen balance in young adults on a protein intake of 0.5 g. per kilogram of body weight, this observation suggests a difference between young and old adults with regard to protein need. Whether these findings mean that an endocrinologically conditioned negative balance can be influenced by raising the protein intake remains at present uncertain.

The reported data from nitrogen balance studies in old subjects show with certainty that the negative balance is not caused by an increase in the total nitrogen loss in the

feces. This does not, however, rule out the possibility that a significant loss of one or more essential amino acids may take place in the feces of old individuals, for instance through incorporation of such amino acids by the fecal bacteria. No research has been performed on this subject, but the advisability for such investigation seems indicated by the finding of a decreased plasma concentration of several of the essential amino acids in aged persons (L. Hofstatter, P. G. Ackermann, and W. B. Kountz, *J. Lab. Clin. Med.* **36,** *259 (1950)*).

The reduction in old age in the volume of active tissue, particularly muscular tissue, is probably one of the most conspicuous causes of the decrease in metabolism with advancing years. The magnitude of the reduction in total metabolism was recently brought out by N. Vinther-Paulsen (*J. Gerontol.* **5,** *331 (1950)*; *Nutrition Reviews* **9,** *304 (1951)*), who measured the actual food intake of 16 debilitated, hospitalized old women. He found that most of these women maintained their weight over a period of months with a daily food intake ranging from 900 to 1250 calories, values which therefore must be considered to represent the total metabolism. As might have been expected a marked inactivity was a conspicuous feature of the clinical condition of these patients.

Investigations on the vitamin nutrition of institutionalized old patients have revealed that the plasma vitamin A levels are not significantly lower than those found in young adults (E. Kirk and M. Chieffi, *J. Nutrition* **36,** *315 (1948)*). Concentrations below 15 micrograms per cent are, however, occasionally seen. It has further been shown (Kirk and Chieffi, *Ibid.* **38,** *353 (1949)*) that the incidence of a subnormal blood thiamine concentration (below 2 micrograms per cent) is about 10 per cent in individuals above 40 years of age, whereas such a low level was not encountered in the study of younger individuals. In both vitamin A- and thiamine-depleted old persons a significant correlation was observed between various clinical signs and the low blood-vitamin level (Chieffi and Kirk, *Ibid.* **37,** *67 (1949)*; *J. Gerontol.* **5,** *236 (1950)*), and it was found that such signs could be favorably influenced by peroral treatment with the specific vitamin and that the administration in most cases was followed by a rise in the blood vitamin concentration to normal (Kirk, *Ibid.* **4,** *327 (1949)*; Chieffi and Kirk, *Ibid.* **5,** *326 (1950)*).

The consistent reports in publications from various countries of a high incidence of low blood ascorbic acid values in old individuals raise the question of the significance of this phenomenon. The low ascorbic acid values have as a rule not been associated with clinical signs of scurvy. In at least some of the groups studied the vitamin C content of the diet was close to the recommended standard for adults, and younger persons consuming the same diet did not show the low ascorbic acid levels (F. Westergaard, "Staseprøven og dens kliniske Betydning," *Ejnar Munksgaard, Copenhagen (1940)*). The possibility therefore exists that the low blood concentration of ascorbic acid in the aged is a physiologic characteristic.

A special interest is attached, in the opinion of the reviewer, to the behavior of thiamine in the intestinal tract. Thus it has recently been shown by J. E. Kirk and M. Chieffi (*Proc. Soc. Exp. Biol. Med.* **77,** *464 (1951)*) that the peroral administration of 5 mg. of thiamine daily in tablet form to both young and old subjects was followed by a marked increase in the thiamine content of the feces, corresponding to a loss from about one-half to two-thirds of the ingested thiamine. This observation is in good agreement with reports of previous studies that with increasing oral thiamine intake a smaller and smaller percentage of the ingested thiamine can be recovered in the urine. It seems likely, with present knowledge, that the conception still widely held that the bacterial synthesis of thiamine

in the intestine constitutes an important supply of this vitamin for the body is not tenable so far as human nutrition is concerned. On the contrary, the considerable loss of thiamine in the feces after thiamine ingestion mentioned above might with more reason be taken to imply that the intestinal bacteria, through incorporation of the vitamin, deprive the body of thiamine administered in the food. The observation in the Elgin State Hospital study (M. K. Horwitt, E. Liebert, O. Kreisler, and P. Wittman, *National Research Council Bull.* **116** (*1948*)) that signs of thiamine deficiency quite regularly developed in old individuals on diets deficient in B-complex vitamins would seem to support the contention that thiamine produced by bacterial synthesis is at least not available for absorption to a significant degree.

It seems likely to the reviewer that the activity of the intestinal bacteria needs closer consideration in future nutritional research. The fact, shown by S. Orla-Jensen, E. Olsen, and T. Geill (*Kgl. Danske vidensk. selsk. biol. skrifter* **3**, *No. 4* (*1945*)), that the intestinal flora of old individuals is both qualitatively and quantitatively different from that of young adults introduces an important gerontologic feature into this nutritional problem.

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INTOLERANCE TO COMPLEX CARBOHYDRATES

Very few metabolic studies have been made of the influence of starch ingestion on fat absorption in the celiac syndrome. For many years, however, it has been customary to treat patients with this syndrome by prescribing diets devoid of complex carbohydrates such as starch.

Studies demonstrating the effect of starch ingestion on fat absorption as measured by twelve-day fat balances have been reported by W. Sheldon (*Arch. Dis. Childhood* **24**, *81* (*1949*)). Fifteen patients, 1 to 6 years of age, presenting findings typical of the celiac syndrome were studied. Each patient served as his own control. The starch-containing diet was similar to the ordinary ward diet and averaged 43 g. of fat daily. The starch-free diet consisted largely of soya bean flour and had a higher fat, protein, and caloric content than the starch-containing diet. The order in which these diets were given was varied. An equilibration period of from one to three weeks on each diet preceded the experimental periods. The results were reported as per cent of ingested fat absorbed.

During the periods in which starch was fed the average fat absorption was 73.2 per cent. When starch was removed from the diet the fat absorption increased to 88.6 per cent. This increase was of high statistical significance.

In a later study (W. Sheldon and A. MacMahon, *Arch. Dis. Childhood* **24**, *245* (*1949*)) it was demonstrated that dextrin, the primary hydrolytic product of starch, produced a similar disturbance in fat absorption. The previously used starch-free diet was found to have a dextrin content of 20 per cent of the total carbohydrate. Fat absorption on this diet was again measured in 4 patients and averaged 90 per cent. When the dextrin content was increased to between 26 and 50 per cent of the carbohydrate, fat absorption by these same patients was reduced to 75 per cent.

More recently these studies have been extended by C. U. Lowe and C. D. May (*Am. J. Dis. Child.* **81**, *81* (*1951*)) to include determination of the nitrogen and electrolyte balance. Four patients with "carbohydrate intolerance" and one normal

infant were studied for a total of 17 four-day metabolic periods. The findings of Sheldon (*loc. cit.*) were confirmed. It was further noted that the defect in fat absorption during the periods of complex carbohydrate feeding persisted in spite of the concomitant administration of crude liver extract and "B" vitamins including folacin.

In the one patient with "carbohydrate intolerance" in which sodium, chloride and potassium balances were also measured, the pattern of balance during the period in which there were no complex carbohydrates in the diet was similar to that found in the normal child. The defect in fat absorption found in this patient was less extreme than that of the other 3 patients studied. The addition of cereal to the regimen altered the pattern of electrolyte retention. During the second cereal period there was an increase in both sodium and chloride retention, relatively greater for chloride than for sodium. These authors suggest that, if this excess chloride remained in the extracellular fluid, sodium would have to leave the cells in order to maintain the physiologic equilibrium. The intracellular sodium would then be replaced by potassium. That this actually happened was suggested by excess potassium retention beyond that theoretically expected on the basis of the nitrogen balance. Good agreement between actual and theoretical retentions of nitrogen as calculated from the potassium balance was found during the period when the cereal was replaced by glucose as well as in the studies on the normal infant. This single observation is of particular interest since disturbances in water and electrolyte metabolism are a well known characteristic of the celiac syndrome.

These observations would suggest that intolerance to complex carbohydrates may play a major role in at least some of the patients having the celiac syndrome. Furthermore, the presence of complex carbohydrates in the diet may disturb the electrolyte pattern of these patients as well as their fat absorption.

FAT AND BLOOD COAGULATION

The relationship between food factors and blood coagulation in clinical conditions is acutely important in patients with gastrointestinal bleeding, usually originating from peptic ulcers. Whether or not such patients should be fed during the period of active hemorrhage has been the subject of much debate among gastroenterologists, with little evidence offered by either side except for data pertaining to survival and morbidity rates under certain therapeutic regimens. Pertinent to this subject is a study of the effect of cream on blood coagulation in humans (J. M. Waldron, B. Beidelman, and G. G. Duncan, *Gastroenterology* **17,** *360 (1951)*).

These workers have studied the effect of the oral administration of 20 per cent cream from cow's milk on the coagulation time as determined by the saline-wetted syringe and collodion-tube technic. In studies of 100 patients receiving 60 ml. of cream, an average decrease in clotting time of 33.7 per cent was found thirty minutes after feeding and a decrease of 43.7 per cent at sixty minutes. Ten control subjects receiving water showed averages of less than 10 per cent decrease at these intervals. A slow return of clotting time toward normal was found over the next three hours. Unfortunately, no ranges of the values obtained are given, and overlap between clotting times in control and experimental subjects is not mentioned.

Blood obtained from control and cream-fed subjects, which was diluted to 60 and 30 per cent with saline, revealed no decrease in clotting time in the latter blood in contrast to the early decrease and subsequent increase in clotting time found in normal blood. Thus it is suggested that the blood of cream-

fed subjects has the characteristics of hypercoagulable plasma.

Dilution of blood *in vitro* with a 10 per cent solution of cream in saline produced decreased coagulation times suggesting the presence of clot-accelerating substances in the cream. A possible local hemostatic effect of cream is thus demonstrated. This may be related to the presence of thromboplastin in cow's milk which has been reported by M. R. Castex and A. Pavlowski (*Medicina* **4,** *389* (*1942*)). That thromboplastin is not responsible for the action of oral cream, however, is suggested by previous studies of J. M. Waldron and M. H. F. Friedman (*Fed. Proc.* **7,** *130* (*1948*)). In studies of heparin and lipemia in animals these investigators found that corn oil administered orally produced an antiheparin effect. In addition, G. G. Duncan and Waldron (*Tr. Assn. Am. Phys.* **42,** *179* (*1949*)) demonstrated that corn oil and olive oil, as well as cream, given orally produce decreased coagulation times in humans. Although these findings suggest that the acceleration of the coagulation reaction is due to fat rather than to other factors present in cream, it would seem wise to reserve conclusions until more data are available, in view of the large number of substances which have been shown to possess thromboplastic activity.

The possible mechanism responsible for the decreased coagulation times following fat ingestion is not suggested by these workers. However, it is noteworthy that similar findings have not been reported by those investigating the use of intravenous fat emulsions for parenteral feedings (S. W. Gorens, R. P. Geyer, L. W. Matthews, and F. J. Stare, *J. Lab. Clin. Med.* **34,** *1627* (*1949*); H. S. Collins *et al.*, *Ibid.* **33,** *143* (*1948*)). These workers have reported the extensive use of several types of fat emulsions including coconut oil, corn oil, butter fat, and mineral oil in either humans or animals, but apparently have not noted changes in clotting sufficiently striking for comment. This discrepancy deserves further study, and no suitable hypothesis can be applied in view of the present confusion as to the chemical nature of thromboplastin and other clot-promoting substances found in plasma.

The observations reported by Waldron, Beidelman, and Duncan (*loc. cit.*) not only suggest a rationale for the use of early fat feeding in the treatment of gastric bleeding, but also lead to conjecture concerning the possible harmful effects of high fat diets in patients with coronary artery or thromboembolic disease. In view of these ramifications of the possible significance of dietary fat in blood coagulation, extension of these studies is clearly indicated.

HEAT DAMAGE TO EDIBLE OILS

Tremendous quantities of vegetable oils are currently manufactured into such edible products as margarine, salad oils, and cooking oils. Since many of these products are, through routine usage, subjected to varying amounts of heat, it is of importance to determine whether or not heat treatment has any effects that would influence the nutritional value of such products.

E. W. Crampton, F. A. Farmer, and F. M. Berryhill (*J. Nutrition* **43,** *431* (*1951*)) of McGill University, Canada, have recently investigated the effect of heat treatment on the nutritive value of herring oil and some commonly used vegetable oils, namely, linseed, soybean, rapeseed, corn, and peanut. The oils were heat-polymerized at 275° C. for periods ranging from 0 to thirty hours in an all-glass apparatus in an atmosphere of carbon dioxide.

The effect of heat treatment on the nutritive values of the oils was measured by the growth rates of young rats that received the oils at dietary levels of 10 or 20 per cent.

Three to 8 animals of each sex were used per treatment and were allowed feed and water ad libitum during the twenty-eight-day test period.

The basal diet consisted of the following, in per cent: casein, 11.5; milk powder, 19.0; bone meal, 2.0; salt, 0.5; oil, 10.0 or 20.0; and white flour to equal 100. The diet was supplemented either with 3 per cent of yeast, or with 5, 10, and 230 micrograms per gram of riboflavin, thiamine, and niacin, respectively. The basal mixture was prepared for feeding by baking at 375° F. for approximately twenty minutes. It was then granulated, air-dried, and mixed with the supplement. Weekly doses of cod liver oil were given orally (175 I. U. of vitamin A and 35 I. U. of vitamin D per animal).

The results indicated that significant decreases in body weight gain resulted with each of the heat-treated oils fed. In general, daily weight gains of approximately 4 g. resulted when the nonheat-treated oils were fed, contrasted with daily gains ranging from 0.8 to 3.4 g. when the heat-treated oils were fed.

The duration of heat treatment of the oils definitely influenced the extent of damage. This was clearly demonstrated with the studies on linseed and soybean oil. For example, linseed oil (10 per cent in the diet) when heated for only two hours permitted body weight gains equal to the controls (3.8 g. daily); four hours of heating permitted weight gains of 3.4 g. daily, whereas eight hours of heating permitted only 2.5 g. average daily gain. Linseed oil heated for twelve hours and fed at a 20 per cent level in the diet resulted in body weight loss.

Heating soybean oil for three hours did not have any significant effect, whereas six and nine hours of heating reduced body weight gains to 3.3 and 2.9 g. respectively when the diet contained 20 per cent of these treated oils. With rapeseed, corn and peanut oils, fifteen hours of heating did not significantly affect body weight gains (10 per cent of heated oils in the diet), but thirty hours of heating resulted in significantly decreased weight gains for all three oils. Herring oil heated for ten hours and fed at a 20 per cent level in the diet produced very poor gains (0.089 g. daily).

Whenever heat treatment of the various oils was severe enough to depress growth significantly, the animals excreted dark, sticky feces. Oily matted coats were noted on the animals fed the heated oils at a 20 per cent level in the diet.

Although no data were presented, the authors stated that any adverse effect of heat treatment on the absorption of the oils from the digestive tract was ruled out. Two possible explanations were given as to the mechanism involved in growth depression from feeding heated oils. It was postulated that growth depression occurred as a result of (1) a toxic factor produced in the heat-polymerization, and (2) the destruction of some other essential dietary component by the polymerized oil.

To test the second postulate the heat-treated oils were fed separately by dropper three hours after fasting and five hours before resumption of feeding the remainder of the diet. Such a feeding technic did not influence body weight gains when the unheated oils were fed. The gain per 1000 calories in the animals receiving the unheated oils was approximately 86 g.

In contrast, when the heated oils were fed separately, the rats gained 3.4 g. daily, whereas feeding the heated oil in the diet permitted only 2.7 g. gain daily. The heated oil, mixed into the diet, adversely affected appetite. However, the body weight gain per calorie consumed was equal in the two methods of feeding (71 g. per 1000 calories consumed). The adverse effect of heat treatment of the oils, therefore, was present whether the oil was fed in the diet or separately, thus suggesting the formation of a toxic product.

The Canadian studies further suggest that there is a relationship between the unsatura-

ted fatty acid content of the oil and its susceptibility to heat damage. Linseed oil was most susceptible to heat damage and also contained more unsaturated fatty acids than any of the other oils tested.

It has been suggested that heat-polymerization is a simple means of stabilizing edible vegetable oils against the development of undesirable flavors and odors (A. S. Privett, R. B. Pringle, and W. D. McFarlane, *Oil and Soap* **22,** *287 (1945)*). Whether or not heat-polymerization of edible oils has any adverse effects in human nutrition remains to be established.

Other investigators have observed adverse effects from feeding heat-treated fats to experimental animals. A review of earlier papers was presented (*Nutrition Reviews* **3,** *42 (1945)*) covering the report that heated cholesterol, olive oil, lard, suet or mutton tallow will produce cancers in experimental animals.

GLYCOGEN FORMATION FROM AMINO ACIDS

Gluconeogenesis is one of the established concepts in physiological chemistry, and data secured on the basis either of the excretion of extra urinary glucose or of increased deposition of hepatic glycogen have long been accepted in its support. That certain of the amino acids contribute to the formation of carbohydrate in the course of their metabolism has long been accepted, although it has been stated that quantitatively reliable evidence for this view exists only for alanine, aspartic acid, and glutamic acid (S. Soskin and R. Levine, "Carbohydrate Metabolism," *Univ. of Chicago Press, Chicago (1946)*). Studies with isotopically labelled alanine also indicate a lack of knowledge with respect to the mechanism of transformation of amino acids to carbohydrate (S. Gurin, A. M. Delluva, and D. W. Wilson, *J. Biol. Chem.* **171,** *101 (1947)*).

A recent contribution to the qualitative aspect of glycogen formation from amino acids has employed isotopic labelling in a somewhat different way (L. C. Terriere and J. S. Butts, *J. Biol. Chem.* **190,** *1 (1951)*). It had been shown that when the body water of experimental rats was enriched with deuterium and glucose was subsequently administered, deuterium was incorporated into the liver glycogen formed. From the rate of uptake of heavy hydrogen, conclusions were drawn regarding the mechanism of formation of the glycogen (D Stetten and G. E. Boxer, *J. Biol. Chem* **155,** *231, 237 (1944)*). In the newer studies, adult fasted rats were given deuterium by intraperitoneal injection to produce a level of approximately 1.3 per cent in the body water. After one hour, glucose solution containing the amino acid to be studied was given by stomach tube in repeated doses. Glycine, DL-alanine, DL-isoleucine and DL-leucine were investigated in this way. After three to twenty-four hours, glycogen was prepared from the liver and the deuterium determined in both the glycogen and liver tissue. From these data an "exchange ratio" of deuterium was calculated.

When glucose alone was given, under these experimental conditions, an exchange ratio of 30.7 was secured; when DL-leucine was added to the glucose no significant change in the ratio was observed, a confirmation of the known failure of leucine to form glycogen. On the other hand, alanine, glycine or DL-isoleucine added to glucose enhanced the formation of liver glycogen as did DL-alanine alone. These observations support the concept of the metabolic pool. Glucose, either as a whole or through smaller fragments, enters and leaves the undifferentiated reservoir as do also the amino acids, and in the process there is presumably an exchange so that deuterium emerges as

part of the synthesized glycogen molecule. With this technic of labelling the newly formed polysaccharide, it is possible, as pointed out by the authors, to demonstrate glycogenic potencies of compounds which, if fed separately from glucose, would cause such a slight change in hepatic glycogen as to render interpretation difficult.

EVALUATION OF THE LIPOTROPIC ACTION OF INOSITOL

Inositol is considered to be an essential nutrient belonging to the B group of vitamins (*Nutrition Reviews* **4,** *163, 349 (1946)*). Among its roles in metabolism is that of a lipotropic agent effective in the mobilization of fat from the liver or in prevention of fatty livers. The relative effectiveness of the lipotropic activity of inositol under different experimental conditions has been studied by a number of investigators (J. M. R. Beveridge and C. C. Lucas, *J. Biol. Chem.* **157,** *311 (1945)*; P. Handler, *Ibid.* **162,** *77 (1946)*; C. H. Best, Lucas, J. M. Patterson, and J. H. Ridout, *Biochem. J.* **40,** *368 (1946)*). Conclusions drawn from experiments done in different laboratories have not always been in agreement.

In an effort to resolve certain of these differences, C. H. Best, J. H. Ridout, J. M. Patterson and C. C. Lucas (*Biochem. J.* **48,** *448 (1951)*) have undertaken experiments with statistical evaluation of the lipotropic function of inositol in rats on diets containing graded proportions of fat of either animal or vegetable origin. The animals used were rats of the Wistar strain which weighed approximately 75 g. at the beginning of the experiment. They were fed a basal diet free of fat, deficient in inositol, and containing 8 per cent casein, 12 per cent gelatin and 72 per cent sucrose plus supplements of salts and vitamins. Animals were killed at the end of three weeks and the liver lipids determined.

The mean concentration of total liver lipids in rats fed the fat-free basal diet for three weeks was determined to be 20.1 per cent of the wet weight of the liver. However, there was great variation in concentrations between individual animals with a range of from 7.2 to 31.5 per cent wet weight. In another group of animals fed the basal diet plus 0.3 per cent inositol the mean concentration of liver lipids was 15.1 per cent, a value which was demonstrably and significantly less by statistical tests than that of the inositol-deficient animals. This observation, therefore, confirmed the small but definite lipotropic effect of inositol in animals fed a fat-free diet.

It had been reported earlier that addition of unsaturated fat such as corn oil to the basal fat-free diet obliterated the effect of inositol, whereas saturated fats were without this effect (Beveridge and Lucas, *loc. cit.*). In the experiments of Best and his associates saturated fat added as the saturated triglyceride fraction from beef fat and fed as 2 per cent of the basal diet was as effective as corn oil in inhibiting the lipotropic function of inositol. Moreover, 0.3 per cent inositol in the diet did not influence significantly the concentration of liver lipids in animals which received diets containing 10 per cent of a moderately saturated fat (Crisco) or mixed fats, 10 per cent Crisco and 2 per cent corn oil. It was therefore concluded that saturated fats are just as inhibitory of the lipotropic action of inositol as are unsaturated fats and that the results of the earlier reports are probably an expression of biologic variation since an analysis of variance was not made of the data. Moreover, it is demonstrated that inositol exhibits significant lipotropic action only in animals fed diets free of fat.

Uniformly throughout these experiments the concentration of total liver lipids was

higher in female animals than in males, but the sex differences were significant only in animals fed 2 per cent saturated beef fat or 2 per cent corn oil. The data indicate that female rats fed fat-containing diets deposit more fat in their livers than do male rats. Moreover, greater quantities of fat occur in livers of female rats fed the fat-free and inositol-deficient basal diet. An apparently greater lipotropic effect of inositol in female animals than in males probably results from mobilization of the excess of fat deposited in the livers of animals of this sex. These findings of sex differences in the dietary production of fatty livers and in the effects of inositol make it imperative that all investigations of this type include either animals of a single sex or equal distribution of males and females in all groups studied.

Another consideration which entered as a possible explanation of differences in results of experiments dealing with the lipotropic action of inositol was that commercial inositol might contain an unevenly distributed contaminant effective in protection against fatty livers. Best and his associates offer evidence that this is not true in that similar lipotropic effects were found with inositol which had been recrystallized several times, fractionally distilled after conversion to the hexapropionic ester, hydrolyzed, and further recrystallized as the free hexitol.

It had also been considered that inclusion of fat in diets containing inositol might interfere with absorption of the lipotropic agent. However, it was found that there was little if any difference in lipotropic effect when inositol was administered by injection rather than by ingestion.

Other investigators have attempted to determine the lipotropic potency of inositol as compared to that of other lipotropic agents. In many of these experiments the diets utilized were not free of choline and the effects ascribed to inositol were those observed in the presence of a second agent (G. Gavin and E. W. McHenry, *J. Biol. Chem.* **139,** *485 (1941)*; P. Handler, *loc. cit.*; M. L. MacFarland and E. W. McHenry, *J. Biol. Chem.* **176,** *249 (1948)*). Similarly, many of these experiments have been carried out on animals after a preliminary period of depletion, *i.e.*, with a fat-free, low protein diet devoid of B vitamins. C. H. Best, C. C. Lucas, J. M. Patterson and J. H. Ridout (*Biochem. J.* **48,** *452 (1951)*) have attempted in recent experiments to clarify certain interpretations derived from earlier investigations by assessing the lipotropic potency of inositol unassociated with other lipotropic agents, as well as by determining the effects of variation in the length of periods of feeding a given diet.

Young male rats weighing from 75 to 95 g. were fed the basal depletion diet for twenty-one days. The diet contained 14 per cent protein (10 per cent casein, 2 per cent arachin and 2 per cent fibrin), 80 per cent sucrose, 2 per cent celluflour, plus adequate quantities of minerals, vitamin A and vitamin D. After three weeks on this diet all animals were provided a vitamin supplement containing biotin, folacin, 2-methyl-1:4-naphthoquinone, pyridoxine, riboflavin, thiamine, pantothenic acid, niacin, para-aminobenzoic acid, and alpha-tocopherol in daily amounts about five times those usually considered adequate for rats. Groups of these rats were given various lipotropic supplements, either inositol, choline, or inositol combined with choline. Animals were killed at various intervals on the supplemented diet and the quantity of fat, and of free and total cholesterol in the livers determined. In later experiments fat was added to the basal diet during the refeeding period. The diet then contained 12 per cent fat, including 10 per cent beef fat and 2 per cent corn oil, the fat replacing equivalent weights of sucrose in the diet.

Control animals which received the supplement of vitamins but no lipotropic material following the depletion period exhibited an abrupt increase in intake of food and in the total lipids of the liver. Inclusion of 0.35 per cent of inositol in the diet was

about as effective as a like quantity of choline chloride in preventing the marked increase in total liver lipid during the first seven days of restoration of B vitamins. The combined action of choline and inositol in other groups of rats exerted a distinctly more rapid and greater action than did choline alone during the first week of supplementation of the fat-free diet. These observations would indicate that inositol has a relatively strong lipotropic effect apart and distinct from that of choline during early phases of development of an acute fatty liver. This agent manifested little if any lipotropic action during a further period of three weeks on this diet.

In groups of animals which received the diet containing 12 per cent fat after the depletion period, supplements of choline prevented the marked increase of total liver lipids which occurred in control animals. Inositol was without effect under these circumstances either as adjudged by its influence when fed alone or in combination with choline.

There was rapid and marked accumulation of cholesterol esters in the livers of animals fed the fat-free diet during the period of supplementation with B vitamins but without lipotropic agents. In contrast, the quantity of free cholesterol in the liver remained unchanged. Less esterified cholesterol accumulated in the livers of animals fed fat than in those on the fat-free regimen. The effect of choline in preventing storage of and in mobilizing cholesterol esters was pronounced and greater in the animals fed fat. The lipotropic action of inositol in terms of its ability to prevent accumulation of cholesterol esters was much less marked than that of choline in rats on the fat-free ration and was negligible in those which received fat.

These important papers do much to clarify the nature and importance of the lipotropic action of inositol. It may be concluded that this substance does have demonstrable action quite apart from that of choline, but that in all respects choline is more effective. Aside from the essential nature of inositol as a member of the vitamin B complex it may be questioned whether there is ever a need in normal nutrition for the lipotropic functions of inositol. In the opinion of Best and his associates inositol should not be regarded as a required lipotropic agent, since it has not been found that a diet deficient in this substance will produce signs of lipotropic-agent deficiency, a syndrome occurring when choline or its precursors, methionine and betaine, are absent from the diet.

CAROTENE POTENCY AUGMENTED BY MARGARINE

There are numerous reports in the literature indicating that carotene, under certain conditions, is less effective as a source of vitamin A than preformed vitamin A. This difference is known to be influenced by variations in absorption and conversion of carotene to the vitamin itself. W. C. Russell, M. W. Taylor, H. A. Walker, and L. J. Polskin (*J. Nutrition* **24,** *199 (1942)*) noted that in chickens, carotene absorption was correlated with the dietary level of fat, whereas the absorption of vitamin A was not primarily affected by dietary fat level.

A. C. Curtis and E. M. Kline (*Arch. Int. Med.* **63,** *54 (1939)*) observed that mineral oil ingestion in man lowered blood plasma carotene levels, an indication of interference with absorption. Similar observations were reported by B. Alexander, E. Lorenzen, R. Hoffman, and A. Garfinkel (*Proc. Soc. Exp. Biol. Med.* **65,** *275 (1947)*). It has been reported that xanthophylls decrease the utilization of carotene (*Nutrition Reviews* **7,** *180 (1949)*) and it has been suggested that this may in part account for the low utilization of carotene in many vegetables (*Ibid.* **7,** *288 (1949)*).

According to a recent report by workers at

the University of Southern California (H. J. Deuel, S. M. Greenberg, E. E. Savage, and D. Melnick, *J. Nutrition* **43**, *371* (*1951*)), the biologic value of carotene is augmented when fed in margarines. Margarine possesses the following characteristics that should make it an ideal vehicle for carotene: (1) It furnishes a readily digestible fatty medium to aid absorption. (2) It usually contains emulsifying agents, such as lecithin, monoglycerides, and diglycerides which aid in absorption. (3) It contains primarily hydrogenated vegetable oils that are relatively stable to oxidative rancidity. (4) It usually contains tocopherols, the natural antioxidants, as stabilizing agents.

The California investigators compared the growth-promoting potencies of vitamin A with carotene when these factors were dissolved either in cottonseed oil or in margarine and fed to vitamin A-depleted rats. They noted that the two fat vehicles permitted equal vitamin A (preformed) utilization for growth, whereas carotene, when administered in margarine, produced a growth response superior to that obtained when it was administered in cottonseed oil, even when the latter was supplemented with tocopherols.

The margarine samples used in the tests were made mainly from materials used in commercial production. One sample of margarine was made that contained carotene as the sole source of vitamin A. Other margarine samples were fortified with carotene concentrate, a 30 per cent suspension of carotene in cottonseed oil (90 per cent beta-carotene and 10 per cent alpha-carotene). The concentrates of preformed vitamin A used were either a blend of fish liver oils, or a solution of distilled vitamin A esters.

Multiple-level (three) biologic assays were conducted on most of the samples, using an elaboration of the U.S.P. XIV procedure. A number of multiple-level assays were also conducted on margarines purchased in the open market.

The carotene in the samples was determined spectrophotometrically. Vitamin A in the samples was analyzed by spectrophotometric and colorimetric ($SbCl_3$) methods. Certain tests of the resistance of samples to oxidative deterioration were also made.

The spectrophotometric estimates and the bioassay results were in good agreement when vitamin A or carotene, dissolved in cottonseed oil (containing up to 0.5 per cent added tocopherols), was fed.

Essentially similar results were obtained when vitamin A was fed in margarine. The biologic responses were 93 and 108 per cent of the estimated potency (spectrophotometric) for vitamin A esters and for fish oil blends, respectively. In contrast, the biologic response to carotene when fed either alone or with vitamin A in margarine was considerably above the expected response based on spectrophotometric assay. Growth responses were 128 and 121 per cent, respectively, of expected values for samples containing carotene alone, before and after seven weeks of storage at room temperature. The corresponding values for the product containing both carotene and vitamin A were 1.16 and 1.25 per cent, respectively, before and after a similar storage period.

Commercial samples of margarine yielded similar results. Thus, vitamin A-depleted rats receiving the vitamin A esters in margarine (white margarine) averaged 113 per cent of the expected gain, based on the estimated biologic potency. The rats receiving approximately one-third of the vitamin A activity in margarine, in the form of carotene (yellow margarine), gained an average of 124 per cent of the expected gain, based on the estimated biologic potency. Since, in the yellow commercial margarine, only about 35 to 40 per cent of the vitamin A activity was present in the form of carotene, the authors calculated that the augmentation of biologic response attributable to carotene in margarine was approximately 30 per cent.

A number of *in vitro* studies were made to

gain some information as to the possible mechanism for the greater-than-theoretical availability of carotene when fed in margarine. The margarine oil used in these tests, a stabilized blend of hydrogenated vegetable oils, was more resistant to oxidative deterioration than cottonseed oil. The margarine had a stability value of one-hundred and eighteen hours contrasted with a stability value of only four hours for the cottonseed oil.

Vitamin A and carotene were stable in margarine oil kept at 140° F. for one-hundred and twelve hours (96 and 89 per cent retention for vitamin A and carotene respectively). The retention of vitamin A and carotene in cottonseed oil was 65 and 73 per cent respectively under similar temperature conditions. Vitamin A (preformed or as carotene) was found to be remarkably stable in margarine. Samples stored for seven weeks at room temperature lost only about 10 per cent of their vitamin A potency, and samples stored for sixteen weeks at 45° F. lost only 2 per cent of their activity.

In summary, Deuel and his associates have shown that only carotene responds to the factors in margarine that promote greater physiologic availability for vitamin A-depleted rats. It is thus again emphasized that vitamin content and vitamin availability in a given food material are not necessarily synonymous. These workers offer no explanation for their results. They do, however, make the general observation that carotene is apparently more sensitive than preformed vitamin A to the environmental factors in margarine that are capable of augmenting the response of vitamin A-deficient rats.

IS THE CARIES-PRODUCING POTENTIALITY OF FOOD PREDICTABLE?

Recent studies with experimental animals have pointed out the need for both bacteria and food residues in the oral cavity for the production of tooth decay. A tube-feeding experiment with white rats demonstrated that no carious lesions formed in animals of high caries-susceptibility unless there were accumulations of food on the susceptible surfaces of the teeth (*Nutrition Reviews* **9,** *101* (*1951*)). Similarly, experimental animals raised and maintained under germ-free conditions did not develop tooth decay when fed caries-producing diets (F. J. Orland, *Military Surgeon* **106,** *345* (*1950*)). These studies point clearly to the need for an oral environmental component in the causation of dental caries. However, these studies do not emphasize this aspect to the exclusion of the systemic factors concerned with the structure of the teeth, the quantity and composition of saliva, etc., nor do they point to any one specific theory as to the etiology of dental decay.

In an investigation in which an evaluation of the caries-producing potentialities of foodstuffs was sought, B. G. Bibby, H. J. V. Goldberg, and E. Chen (*J. Am. Dent. Assn.* **42,** *491* (*1951*)) have reported the results of a series of studies in which they determined: (1) the amount of acid produced upon incubation with saliva of a given weight of various common foodstuffs, and (2) the amount of the same foodstuffs retained in the human oral cavity after the normal chewing and swallowing processes. These investigators believed that tooth decay was the result of acid formation from food on the tooth surfaces. They postulated further that the ability of individual foods to produce tooth decay through their oral environmental relationship was a function of the acid produced per unit weight of food and also a function of the amount of food retained on the tooth surfaces. Hence, the "caries-producing potentiality" or the "decalcification potential" of any food was defined by these authors as the units of acid formed from a standard amount of food

upon incubation with oral bacteria multiplied by the units of food adhering to the teeth after eating that particular food alone.

The determination of the amount of acid produced from a foodstuff was begun by mixing 3 g. of the test substance with 100 ml. of distilled water in a Waring Blendor. Aliquots of 5 ml. of this food mixture were added to test tubes containing 5 ml. of paraffin-stimulated saliva. Then a preincubation titration was made on an aliquot with 0.1 normal sodium hydroxide solution. After incubation for four and twenty-four hours, other aliquots were titrated. The differences in milliliters between the 0 hour and the four- or twenty-four-hour titrations were recorded as the values for the acid produced. Only the four-hour value was used in calculation of the "decalcification potential." The amount of acid produced in four hours by this method varied from lows of 0.6 ml. for oil-sprayed crackers and 0.7 ml. for corn flakes and canned pineapple to highs of 3.1 ml. for pretzel sticks and 3.3 ml. for mashed potatoes. The amount of acid produced in twenty-four hours varied from 1.2 ml. for finger-shaped soda crackers, 1.4 ml. for a bran cereal and milk to 3.3 ml. for mashed potatoes, fig bars, and canned peaches, 3.5 ml. for dates and 3.7 ml. for figs.

The amount of carbohydrates and of soluble carbohydrates was determined for each foodstuff. However, there appeared to be absolutely no relationship between either the total carbohydrate or the soluble carbohydrate content of the foodstuffs and the amount of acid produced on incubation with saliva.

The eating process consisted of the subject chewing and swallowing normal mouthfuls of the test substance. After the last swallow, the subject was allowed to clean his teeth with his tongue or by sucking, in whatever he considered to be his normal or habitual procedure. Then the teeth were thoroughly cleaned by tooth brush or by atomizer and all the washings collected. The actual amount of food obtained from the teeth was determined by an indirect procedure. The carbohydrate content of the washings was determined in order that the amount of food represented could be calculated on the basis of the known percentage of total carbohydrate in the original sample. The amount of food substance retained on the teeth varied from 2 mg. for carrots, grape soda and orange soda, 3 mg. for apples, 16 mg. for egg noodles, and 21 mg. for corn flakes, up to 266 mg. for fig bars, 338 mg. for white bread and peanut butter, 350 mg. for figs and 394 mg. for one variety of candy bar. There appeared to be no relationship whatsoever between the total or soluble carbohydrate content of the food and the amount retained on the teeth.

The "decalcification potentials" ranged all the way from 2 for carrots, 15 for corn flakes, 16 for lettuce, 18 for noodles, 25 for cucumber and 26 for oil-sprayed crackers and grape soda up to 504 for dry prunes, 505 for dates and fig bars, 541 for white bread and peanut butter, and 665 for figs.

In an effort to study the extent to which the fat, salt and baking soda content of foods influenced their "decalcification potentials," a basic cake formula in which the composition of the various components could be varied through wide ranges was mixed and baked under standardized conditions in the laboratory. The resultant cakes were used for determination of acid production in saliva and for measurements of the amount of retention on the tooth surfaces. Increases in the fat content appeared to decrease the amount of food retained on the teeth but had no effect on acid production. Hence, the "decalcification potential" was decreased with fat increases. The omission of salt from the formula appeared to increase the retention of food slightly. The addition of baking powder to produce cakes of lighter texture was interpreted to cause slightly lower food retention.

The question of the amount of retention

of any single food in different mouths was considered in what appeared to be a rather casual fashion in spite of the considerable importance of such variation. Eight foods or food mixtures were tested in a total of 22 trials. The retention of white bread varied from 95 mg. to 129 mg. in the 5 individuals studied. Incidentally, this was the only food studied in more than 3 individuals. Six other foods or food mixtures were studied in 2 or 3 individuals each with a variation of 10 to 25 per cent in the amount of food retained on the teeth. However, the retention of cinnamon melba toast was 93 mg. and 278 mg. for the 2 individuals tested. In the computation of its "decalcification potential," the lower value was taken to give a reading of 141, the figure recorded. The use of the higher figure would have increased this value to 417. In view of such a variation one may ask: does either 141 or 417 have any meaning?

In order to test whether there was a relationship between "decalcification potential" and actual production of tooth decay in hamsters, an experiment was conducted to compare graham crackers, oil-sprayed crackers and white bread. These foods were fed with a diet consisting of 60 per cent powdered milk, 10 per cent wheat germ, 5 per cent cellulose, 4 per cent alfalfa and 1 per cent sodium chloride. These diets were fed in unlimited quantities; likewise, no restriction was placed on water consumption. A total of 50 hamsters were used, 2 of which died during the experiment. At the end of eighty-six days, the hamsters were killed and the teeth evaluated for evidence of dental caries. The average weight gain for the hamsters fed the graham crackers diet was 63 g., for those fed the oil-sprayed cracker diet 50 g. and for those fed the white bread diet 25 g. The average caries scores for male hamsters on the graham crackers diet, the oil-sprayed cracker diet and the bread diet were 47, 25 and 7, respectively; the scores for female hamsters were 16, 14 and 5, respectively. The "decalcification potentials" for graham crackers, oil-sprayed crackers and white bread were determined to be 226, 26 and 164, respectively. In other words, even though there was an 8:1 ratio of the "decalcification potentials" of graham crackers to oil-sprayed crackers, the ratio of caries scores for males was about 2:1 and for females was about 1:1. These ratios were considered by the authors to give "some support for the hypothesis that the decalcification potentials are an index of their caries-producing potentialities." Although the ratio of the "decalcification potentials" of oil-sprayed crackers to white bread was 1:6, the ratio of caries scores for males was about 4:1 and for females was about 3:1. Although the investigators failed to comment, the latter comparison seems to be particularly damaging to their premise that the "decalcification potentials" of foodstuffs are related to their caries-producing potentialities.

It is obvious from the tabular material presented in this paper that a great deal of time and effort have been expended in the collection of these data. Furthermore, it is readily evident that the authors believed implicitly the theory of the etiology of dental caries around which they have planned their experiments. However, neither their experimental data nor their discussions of the results justify any optimism that it has been possible to evaluate the caries-producing potentialities of various foodstuffs as proclaimed by the title of the paper. On the contrary, their results with hamsters where graham crackers, oil-sprayed crackers and white bread were compared seemed to point strongly to the lack of any correlation between "decalcification potential" and caries production in the hamster.

There are a number of questions which arise about the experimental procedures concerned with the collection of the tabulated data on the "decalcification potentials" of foodstuffs. The first and possibly most important is the individual variation in retention of foods on the tooth surfaces. This has already been mentioned above with a

summary of the few results presented and the high variations from individual to individual. Would there be similar variations in successive trials with the same individual, or did the variation between individuals occur because of different tooth anatomy and arrangement? No information was given on this point. Indeed, there is no comment on how many individual tests were averaged to give the "decalcification potential" for any foodstuff. Or was each value the result of a single trial? If the latter surmise is correct, were all the values for the 96 foods listed in tables 4 to 10 determined as the result of retention studies in one individual or were a number of individuals concerned? A critical evaluation of this paper requires answers to these questions in order to judge the scientific care and accuracy used in the compilation of the results.

The paraffin-stimulated saliva used in the acid production studies needs some further description. The chemical composition of saliva and the number and distribution of oral bacteria therein differ widely from individual to individual and within the same individual at different times. The amount of acid production depends upon the flora and the substrates in the saliva as well as upon the composition of the foodstuff. Therefore, if the results are to vary only by reason of the foodstuff added to the saliva, there must be some standardization of the saliva. However, again no comment was made as to how this was accomplished or, indeed, whether standardization was attempted.

On the basis of the experimental data presented in this paper, no reasonable conclusions as to the caries-producing potentialities of any of the 96 foodstuffs can be made. Providing that some of the questions asked above as to the scientific validity of the procedures can be answered satisfactorily, the "decalcification potentials" may become a general guide for the setting up of animal experiments to test the relation of food retention in the oral cavity to the real caries-producing potentiality of foods. Despite the intensiveness of this research project, there is yet no known shortcut via *in vitro* tests to substitute for animal experimentation on dental caries.

VITAMIN E LEVELS IN NEWBORN INFANTS

Recent studies suggesting the use of tocopherols in the treatment of retrolental fibroplasia (*Nutrition Reviews* **8,** *116 (1950)*) have stimulated interest in the vitamin E nutriture of newborn infants, particularly those born prematurely. Although no evidence favoring a relationship between serum tocopherol concentration and the subsequent development of this disease has been presented, the blood of these infants contains less vitamin E than that of adults. Interpretations based on these findings and their possible significance to the physiology of the infant have awaited studies of the serum tocopherol concentration in normal infants and elucidation of the factors associated with its variations.

The microchemical method for the determination of serum tocopherols developed by M. L. Quaife, N. S. Scrimshaw, and O. H. Lowry (*J. Biol. Chem.* **180,** *1229 (1949)*) has been appropriately applied to such studies. A total of 191 determinations on 53 full term and 32 premature infants, all of whom had an uncomplicated hospital course, has been reported by W. T. Moyer (*Pediatrics* **6,** *893 (1950)*). The cord blood obtained from 33 full term infants had an average concentration of 0.22 mg. per cent with a standard deviation of 0.13. This value was not statistically different from the average concentration of 0.24 mg. per cent found in venous blood on the first day of life in the same group of infants. When both cord and venous blood values were pooled, an average of 0.24 mg. per cent was found

for the entire group of 53 full term infants.

An average concentration of 0.22 mg. per cent was found in a group of 11 premature infants studied during the first day of life. Further analysis of these data by weight groups did not reveal significant differences in association with birth weight.

Similar results are reported by S. W. Wright, L. J. Filer, and K. E. Mason (*Pediatrics* **7,** *386* (*1951*)). Serum tocopherol determinations were performed on 77 term infants on the first or second day of life before they had received their first feeding. They reported an average value of 0.38 mg. per cent with a standard deviation of 0.18. Thirty observations on 21 premature infants between 1 and 10 days of age averaged 0.43 mg. per cent. The small difference between these groups was not statistically significant. Although these values are higher than those reported by Moyer for the first twenty-four hours of life, they are comparable with the average concentration of 0.36 mg. per cent which he found for a group of 19 full term infants between 2 and 5 days of age. The type of feeding received by these infants was not reported.

At the time of parturition the serum tocopherol concentration of mothers is increased to almost twice the normal adult value (N. S. Scrimshaw, R. B. Greer, and R. L. Goodland, *Ann. N. Y. Acad. Sci.* **52,** *312* (*1949*)). On the second postpartum day determinations performed on the mothers of the infants reported by Wright, Filer, and Mason averaged 1.82 mg. per cent for those who later nursed their infants and 2.05 mg. per cent for those who did not. This difference was not statistically significant. The difference between infant and maternal values was considered confirmation of the fact that placental transfer of this vitamin is decidedly limited. Similar conclusions had previously been reached on the basis of animal experiments (K. E. Mason and W. L. Bryan, *J. Nutrition* **20,** *501* (*1940*)).

The possibility that mammary transfer of vitamin E is quantitatively important was also investigated. Serum tocopherol determinations were performed on the second, fourth and sixth day of life and at 1 to 4 and 5 to 8 months of age on a group of 40 nursing infants and a comparable group of artificially fed infants. No difference between these groups was apparent immediately following birth. By the sixth day of life the breast-fed infants were found to have tocopherol levels averaging 1.46 mg. per cent and remaining above 1.25 mg. per cent during the period of observation. The artificially fed infants had significantly lower values averaging 0.50 mg. per cent on the sixth day and reaching a value of 0.75 mg. per cent only in the 5- to 8-month period. This difference is apparently due to the tocopherols supplied by the colostrum of human milk. This food has been found to contain as much as 3.6 mg. per cent (M. L. Quaife, *J. Biol. Chem.* **169,** *513* (*1947*)). Cow's milk formulas and human milk after the eighth day usually contain between 0.10 and 0.15 mg. of tocopherols per cent.

In a group of premature infants receiving a low-fat formula containing only 0.04 mg. tocopherols per cent a steady decline was noted from the original concentration of 0.43 mg. during the first 10 days of life to an average value of 0.09 mg. per cent between 31 and 40 days of age.

Great care should be exercised in interpreting these findings. The premature infant fed a formula low in tocopherols rapidly develops a low serum tocopherol level which may approach that associated with typical deficiency symptoms in the young experimental animal. Low values in humans are usually seen in association with defects in fat absorption. Poor fat absorption is also characteristic of prematurity (*Nutrition Reviews* **8,** *349* (*1950*)). As yet no dysfunction of infancy has been related to a deficiency of vitamin E. Conclusions concerning the relationship between serum tocopherol concentrations and the health of these infants are not warranted at the present time.

AGE FACTOR IN CHOLESTEROL-INDUCED ATHEROSCLEROSIS IN CHICKS

Observations made of chickens have demonstrated spontaneous occurrence of atherosclerosis comparable to that which develops commonly in man. D. V. Dauber described arteriosclerotic lesions in 45 per cent of chickens over one year of age which he examined (*Arch. Path.* **38,** *46 (1944)*). The susceptibility of this species to atherosclerosis was further demonstrated when it was found possible to produce lesions consistently by inclusion of cholesterol in the feed (Dauber and L. N. Katz, *Ibid.* **34,** *937 (1942)*). In contrast to other species, particularly the rabbit and dog in which atheromatosis may also be induced experimentally by dietary means, only in chickens and in other birds does the pathologic entity occur spontaneously under natural conditions of diet and environment (*Nutrition Reviews* **8,** *74 (1950)*). It would seem, therefore, that the chicken is particularly well adapted for the laboratory investigation of those factors which pertain to and condition the occurrence of atheromatous changes.

The concept is held by some that atherosclerosis is a simple manifestation of aging, that its occurrence is to be considered a normal event resulting from repeated injury to the arterial wall with increased formation of fibrous connective tissue and changes in the ground substance (A. I. Lansing, M. Alex, and T. B. Rosenthal, *J. Gerontol.* **5,** *315 (1950)*). In a recently reported investigation S. Rodbard *et al.* (*Circulation* **3,** *867 (1951)*) have attempted an evaluation of the effect of age and the aging process on the susceptibility of chicks to atheromatosis and hypercholesteremia when diets were supplemented with cholesterol. An interesting and unanticipated observation was made when it was found that certain age periods in the life of the chick are characterized by increased susceptibility to hypercholesteremia and the arterial lesions.

One-day-old cockerels were utilized in this study. They were immediately placed on the experimental diet composed of a starter mash to which was added 2 per cent commercial cholesterol and 5 per cent cottonseed oil. Another group of chicks served as controls and received starter mash without supplements. A number of chicks from each group were killed at the initiation of the experiment and at frequent intervals up to an age of 26 weeks. The animals were carefully examined after sacrifice for gross evidences of aortic atheromatosis and organ lipoidosis. Atherosclerotic lesions in the thoracic aorta were graded according to a system utilized in this laboratory for a period of years. Blood specimens were obtained at frequent intervals for determination of concentrations of cholesterol in plasma.

Much higher concentrations of cholesterol in plasma were found on the day of hatching than in later weeks in chicks fed the control diet of starter mash. The average concentration of cholesterol in plasma at birth was about 300 mg. per cent. This level decreased during the first weeks of life to about 100 mg. per cent where it remained without major fluctuations for the first twenty-six weeks of life. The high level of plasma cholesterol at birth is explained on the basis of absorption and metabolism of yolk material which is enclosed within the abdomen shortly before hatching and which contains relatively great concentrations of cholesterol.

The same changes in plasma cholesterol did not occur in chicks fed the diet containing cholesterol and cottonseed oil. Rather, the high concentration present on hatching persisted or decreased only slightly during the first five or six weeks. Then there occurred a marked increase from the eighth to tenth week. This high level then persisted through the next twelve weeks after which concentrations of cholesterol in plasma spontaneously decreased but never reached the much lower values observed in the control animals. In one experiment, chicks on the

diet supplemented with cholesterol and cottonseed oil had a mean plasma cholesterol of 192 mg. per cent at five weeks, 505 mg. per cent at ten weeks, 822 mg. per cent at twenty weeks, and 262 mg. per cent at twenty-five weeks. Experiments run at different times of the year, *i.e.*, August and February, did not give quantitatively similar results in terms of the changes in plasma cholesterol, although the direction of the changes remained the same. The authors, therefore, suggest that factors of climate or season may possibly influence systems of cholesterol metabolism in the chick on the high cholesterol intake.

In earlier experiments designed for other purposes, chicks had been placed on the high cholesterol diet not at hatching but at from 4 to 7 weeks of age. There had been considerable, unexplained variation in plasma cholesterol in these animals. When these data were re-examined by the authors it was noted that regardless of a variety of other experimental conditions there was impressive regularity of occurrence of a marked rise in cholesteremia at about the eighth week of life.

To examine this phenomenon further, groups of chicks were started on the diet supplemented with cholesterol and cottonseed oil on hatching and at 5 or 7 weeks of age. In each group of animals the mean plasma cholesterol had increased to nearly 1500 mg. per cent by the tenth week of life. In the groups started at 5 and 7 weeks this represented an increase of about 1400 mg. per cent within five and three weeks of cholesterol supplementation respectively.

Therefore important evidence seems to have been presented which indicates variation in metabolism and utilization of cholesterol which is directly related to the age period of 8 to 10 weeks in the chick. Moreover, this mechanism is not detected unless cholesterol is fed, but is independent of exposure to cholesterol before the seventh week.

This observation took on added significance when it was determined that there was a direct relationship between the occurrence of high levels of plasma cholesterol and the appearance of atheromata in the thoracic aorta as well as in the brachiocephalic and coronary vessels. In one experiment, the first arterial lesions were noted beginning in the eleventh week. Thereafter the percentage of animals with thoracic atheromatosis increased as did the severity of the lesions. At the end of fifteen weeks all birds killed were found to have typical lesions.

There was a total of 381 chicks utilized in these studies. However, small groups of birds were used in certain parts of the experiments. The reader would have been greatly assisted in his evaluation of the report if the data had been subjected to statistical analysis. Perhaps the authors felt that the constancy of the results obviated the necessity for such analysis. In truth there is little reason to doubt that an analysis of variance would substantiate the conclusions drawn, but certainly such an evaluation should have been made.

The approach utilized here is one of the utmost importance in the study of those diseases considered to be degenerative. It considers factors under control of the organism itself. That endogenous changes in metabolism are associated with the age of the animal is well demonstrated in these data. Further, evidence is presented that the metabolic state may condition the response of the animal to exogenous factors which are under experimental control. It is of interest that rabbits under one year of age are less susceptible to experimental atherosclerosis than are older animals (O. J. Pollak, *Arch. Path.* **43,** *387 (1947)*). Again, the disease in humans is most commonly associated with later life, and atheromata occurring in early life are usually associated with other metabolic diseases or defects. It is conceivable that those factors which are important in the causation of atherosclerosis in the human are conditioned in their effect by metabolic variations associated with age.

NUTRITION AND INDUSTRIAL OUTPUT

In 1944 E. Simonson noted that no really well controlled investigations on the effects of different diets on industrial output have been reported (*Ann. Rev. Physiol.* **6,** *543 (1944)*). The need for such studies has been repeatedly emphasized (Council on Foods and Nutrition and Council on Industrial Health, *J. Am. Med. Assn.* **118,** *618 (1942)*). F. G. Boudreau (in Introduction, "Nutrition in Industry," *International Labor Office, Montreal (1946)*) pointed out the sore need for controlled studies which would throw light on the influence of diet on health and work output. In this country a limited amount of experimental work was carried out on some aspects of industrial nutrition, such as the effectiveness of vitamin supplements (*Nutrition Reviews* **8,** *312 (1950)*) and between-meal feeding (*Ibid.* **8,** *167 (1950)*).

In Germany an opportunity to study the relationship between caloric intake and performance presented itself during the Second World War (summer of 1944) when some 3500 Russian and Italian workers employed in the iron industry were given food supplements in an effort to raise their output. The details of this mass experiment were reported by H. Kraut *et al.* (*Arbeitsphysiol.* **14,** *147 (1950)*).

At the start, the average daily caloric intake in the three categories of work, graded according to energy expenditure, was 2500, 2900, and 3700 calories for the Germans; 2300, 2600, and 3000 calories for the Russians; and 2600, 2800, and 3100 calories for the Italians. In July the intake of the foreign workers was increased by 300 to 400 calories. Additional increases raised the level to 3100, 3300, and 3600 calories for the Russians and to 3200, 3400, and 3700 calories for the Italians. The period of observation ended in December 1944. It is not clear what happened to the caloric intake of the German workers during this time.

Adequate records of the health status and body weight were available for about 1000 workers. In a sample of workers falling into each of the three categories of energy expenditure, performance records were kept during the whole experimental period of six months.

The effects of the supplements were dramatic. Expressed in terms of the norm for German workers during peacetime, the performance of the Russian workers rose from the initial value of 70 per cent to a value of 84 per cent in December of 1944; the average output of the Italians increased from 77 per cent in June to 93 per cent in December. There was an average increment of body weight of 3.0 and 2.5 kg., respectively. In a camp of 586 Russian and 403 Italian workers the sickness absenteeism rate in July was 13.5 and 14.5 per cent, respectively, as compared to the sickness rate of the German workers in the two plants of 6.7 and 4.5 per cent. In the second plant, employing Italian and German workers, the sickness rate among the Italians decreased steadily until it reached 4.9 per cent while in December of the same year the German workers had a sickness rate of 7.4 per cent. In the other plant the change was less marked, with a decrease from 13.5 to 12.1 per cent. It should be noted that the sickness rate for the German workers *increased* from 6.7 to 9.1 per cent. These comparisons suffer, in part, from the fact that the age and fitness of the foreign and German workers were not matched, the former being considerably younger. Another complicating factor was an influenza epidemic which was at its height at the beginning of the study. Especially for the Italian workers this made the initial sickness rates unrepresentatively high and the relationship between the increased food intake to decreased sickness rate spuriously close. A reliable relationship has been demonstrated between nutritional deficiency symptoms ("hunger edema" and exhaustion) and improved nutrition of the Russian workers. The number of patients with such

symptoms decreased monthly from 65 in July to 46, 34, 19, 23, and 11 in December. In the Italian group edema was very infrequent even at the start of the experiment.

As would be expected on physiologic grounds, the improvement in performance was not the same in different types of work. In some jobs, such as operating blast furnaces or presses, the production process was fully mechanized and the speed of the particular piece of equipment, such as a press, the frequency of breakdown, and the supply of material were the factors limiting performance. The human factor played a subordinate role. Consequently, little rise in output could be expected following improved nutrition and little change was actually observed. On the average, 55 Italian and Russian workers falling into this job category increased their output, expressed in percentage of the prewar German norm, from 77.5 per cent to 81.1 in the middle of the experimental period and to 83.5 per cent toward its end. At the same time the workers on these jobs showed the largest weight increments, with average weights of 60.7, 62.3, and 64.0 kg. While the output increment was relatively small, the improved physical and mental status was reflected in increased endurance and in carrying out accessory work activities which were neglected before.

In the second group of industrial jobs, *e.g.*, lathe work and operating some types of drill presses, the output depended both on the machine and the human operator. This group of 97 workers showed a somewhat larger output increment (averages of 73.3, 84.4, and 87.1 per cent of the norm) but lesser weight increment (59.4, 61.4, and 62.1 kg.) than the first group.

In the third group, including such operations as grinding done by hand, filing, and welding, the output was directly related to the capacity and effort of the individual worker. There was a very marked rise in output (70.1, 85.7 in the mid-period, and 89.0 per cent in the end period) while the weight, after an initial rise, became stabilized (averages of 60.9, 63.4, and 63.4 kg. for 185 men).

The authors point out that a prolonged caloric deficit will affect the output in a progressively larger number of occupational activities and will finally include those operations in which the man acts essentially but not completely as a machine tender.

Knowing the initial caloric intake and the industrial output, as well as the effects of increased food intake on body weight and output, the authors undertook to estimate the caloric needs of 25 jobs in the iron industry under conditions in which the output would equal the prewar norm. The estimate of daily energy requirements ranged from 2950 to 4460 calories, while the caloric cost of the occupational activities varied from 750 to 2300 calories.

G. Lehmann, E. A. Müller, and H. Spitzer (*Arbeitsphysiol.* **14,** *166 (1950)*) stressed that such an extrapolation is reasonably accurate only when the differences between the actual output and the norm (100 per cent output) are not too large. When the output decreases to some 50 per cent, an extrapolation is not permissible.

Lehmann *et al.* (*loc. cit.*) undertook a task opposite to the one just cited and attempted to estimate the probable reduction in output resulting from reduced food intake. Their premise is that the workers almost automatically will reduce the physical effort in order to preserve an equilibrium between energy expenditure and energy intake. Separate prediction tables were worked out for individuals engaged in occupations with a low to very high energy output (2400, 3000, 3600, and 4200 calories for a 100 per cent performance). The fact that basal metabolism is lowered in the course of prolonged undernutrition was taken into account and it was postulated that the caloric expenditure for nonoccupational activities will also decrease. The main data are summarized in Table 1.

The estimates of the amount of deterioration appear at first sight fairly reasonable

for the jobs requiring high energy expenditure, but exaggerated for light work. Principal potential sources of error are the values of basal metabolism during undernutrition, which are probably too high. This results in very low values of calories available for occupational activity. It should be noted also that light industrial occupations involve either visual inspection, mental work, or manipulative skill. It has been established that visual acuity is not affected by a prolonged starvation, that mental capacity shows a remarkable resistance to deterioration during nutritional stresses, and that coordination and manual speed are much less affected than muscular strength and endurance (A. Keys *et al.*, "The Biology of Human Starvation," *Univ. of Minnesota Press, Minneapolis (1950)*). However, the fact that work output is a product of work capacity and work effort, and that the two components may vary to some extent independently of each other should not be lost from sight.

TABLE 1

Estimated deterioration of output at different levels of caloric deficit

LIGHT WORK		MODERATELY HARD WORK		HARD WORK		VERY HARD WORK	
Cal.	% Output	Cal.	% Output	Cal.	% Output	Cal.	% Output
2400	100	3000	100	3600	100	4200	100
2200	65	2750	76	3300	82	3900	88
2000	30	2500	58	3000	66	3600	76
1750	—	2250	42	2750	54	3300	64
1500	—	2000	27	2500	44	3000	55

Perhaps the absence of a time parameter in the table of Lehmann *et al.* (*loc. cit.*) is the single most important deficiency of the table. The longer the duration of undernutrition at the same caloric deficit, the greater—within limits—is the reduction in basal metabolism which represents a most important single physiologic mechanism of adaptation to the dietary energy deficit.

A rough test of the accuracy of prediction may be found in the data presented by Kraut *et al.* (*loc. cit.*) In highly mechanized occupations, the average caloric intake for full productivity was estimated at about 3100 calories. At an intake of 2500 the output of the Italian workers was 85 per cent, that of the Russian workers 76 per cent of prewar German standards, and not about 55 per cent, as would be predicted from the table constructed by Lehmann *et al.* This suggests that for moderately hard work the table overestimates the drop in productivity.

The two categories of "hard work," with standard energy requirements of about 3300 and 3900 calories, may also be considered. At intake levels of 2800 and 2600 calories, and 3100 and 3000 calories for Italian and Russian workers respectively, the average performance rates were 75.1 and 72.2 per cent in the first of the two categories, and 76.7 and 65.8 per cent in the latter category. The output values obtained by interpolation from the table would be roughly 65 and 54, 68 and 69 per cent, respectively. Throughout, the discrepancies were in the same direction; quantitatively, the discrepancy was largest for the category of moderate work. The magnitude of the discrepancies may have been somewhat exaggerated by the smaller body size of the Italian and Russian workers. This factor is not considered in the table but it entered the calculations of caloric needs for 100 per cent output.

Lehmann *et al.* have attempted to answer a practical and frequently asked question. If brought into line with other known facts and revised downward, together with addition of information about the duration of the caloric deficit, the table may serve as a useful guide for gauging the effects of imposed prolonged food restrictions.

COMPARATIVE INTESTINAL ABSORPTION OF D- AND L-ISOMERS OF AMINO ACIDS

The D- and L-isomers of the amino acids are known to follow different metabolic pathways within the body (*Nutrition Reviews* **4,** *173 (1946)*; **8,** *118, 202 (1950)*). Little information is currently available concerning comparative rates of absorption of these isomers from the intestinal tract. A report dealing with this type of investigation is, therefore, received with interest. Such a study has recently been reported by Q. H. Gibson and G. Wiseman of the University of Sheffield, England (*Biochem. J.* **48,** *426 (1951)*). The procedure utilized by these investigators was to determine by stereochemically specific methods the disappearance of D- and L- forms of various amino acids from loops of small intestine in the rat.

The intestinal loops were prepared by abdominal operations on adult rats under nembutal anesthesia. The ileocecal junction was identified and approximately 15 cm. of small intestine proximal to this area was utilized for construction of the loop. One end of loop was tied off and the segment of intestine washed out with distilled water. Isomers of an amino acid contained in one ml. of solution were then instilled into the loop and the other end of the loop secured by ligature. The intestine was then replaced in the abdomen without interference of its blood supply, and the abdominal incision sutured. The rats were maintained at body temperature and were killed after selected intervals of time. The loop of intestine was removed, its length determined and its contents washed out with distilled water.

The analytic methods adapted for this study were largely manometric methods utilizing specific enzymes. Among the enzymes prepared and used were L-amino acid oxidase of *Neurospora crassa*, bacterial decarboxylases for L-histidine, L-lysine, and L-glutamic acid, and D-amino acid oxidase of pig kidney. When only one isomer was determined by a specific method, the value for the other was obtained by difference, after determination of total amino acid with chloramine-T.

In preparation of the intestinal loops an attempt was made to keep the lengths constant at 15 cm., since it was thought that variation in loop length would influence the rate of absorption. However, disappearance rates of amino acids from loops of intestine varying about 25 per cent from standard lengths could not be shown conclusively to be affected by the length of the loop.

In an effort to ascertain proportions of a given isomer absorbed from intestinal loops when different quantities were instilled into the loops, the amount of amino acid introduced was varied. Experiments with L-glutamic acid and L-histidine demonstrated that the proportion of each isomer absorbed within one-half hour was about the same with introduction of from 60 to 225 micromoles of the amino acid. In all other experiments, therefore, the amount instilled was kept constant at levels from 150 to 250 micromoles for any one amino acid, and equal quantities of the D- and L-isomers were placed in the loops.

Disappearance rates during periods from one-half to one hour of D- and L-isomers of thirteen amino acids were determined. Included were the isomers of alanine, phenylalanine, methionine, histidine, valine, leucine, isoleucine, norleucine, aspartic acid, glutamic acid, α-aminoadipic acid, α-aminopimelic acid, and lysine. In all instances significant absorption of the L-isomer occurred and proceeded at a more rapid rate than that of the D-form. Absorption ratios comparing L- and D-isomers varied from 1.6 for methionine to 6.0 for histidine. Inherent in the data recorded is considerable variation in percentage disappearance of the L-isomers of different amino acids. The authors have chosen not to comment on these dif-

ferences, probably because of variation in length of intestinal loops, in length of experimental periods, and in quantities of the amino acids introduced.

As a control the investigators determined the disappearance of stereoisomers of certain of these amino acids from intestinal loops of animals killed just prior to instillation of amino acid mixtures. In all instances the quantity of a given isomer which disappeared was insignificant.

Gibson and Wiseman (*loc. cit.*) interpret their observations as providing evidence of a specific mechanism for the active absorption of L-amino acids, since if absorption were by a process of simple diffusion the rate of disappearance of both D- and L-isomers would be the same. They consider it unlikely that their results can be interpreted as contingent on bacterial action since each loop was carefully washed out before the solutions of amino acids were introduced and since there were no striking differences in disappearance of amino acid isomers from the intestinal loops in dead animals. They also deem it unlikely that amino acids are oxidized in the intestine, since the intestinal mucosa has not been found to be a rich source of amino acid oxidases.

Limitations of these experiments reside in the fact that these animals were anesthetized and subjected to rather severe surgical manipulation with ligatures applied at two points along the course of the small intestine. However, the observations are consistent in demonstrating a faster rate of disappearance of the L-isomer as compared to the D-form of all the amino acids studied. Important confirmatory information might be achieved by demonstration of greater quantities of the L-isomer in intestinal lymph or venous blood from mesenteric vessels.

The approach utilized is one of interest, and may provide a method of further study of mechanisms of intestinal absorption. The evidence achieved certainly favors the concept of an active mechanism of selective absorption of amino acids rather than one of simple diffusion through mucosal barriers.

NORMS AND VALIDITY OF MEASURES OF RELATIVE LEANNESS-FATNESS IN MAN

Availability of norms based on standardized and valid methods of measuring body fat is a prerequisite for a precise classification of individual subjects along the leanness-fatness continuum, for interpretation of data provided by nutritional surveys, and for longitudinal investigations of calorie nutriture of the population in times of food shortages and rationing. For children, some normative data are available. Thus H. V. Meredith and H. C. Stuart (*Am. J. Pub. Health* **37,** *1435 (1947)*) provided values of skinfold thicknesses corresponding to the clinical ratings, on a five-point scale, of the amount of subcutaneous tissue below the scapula and above the crest of the ilium. The basic data were obtained during 1930 to 1945 on Iowa City children of northwestern European ancestry. Separate tables are provided for boys and for girls. The age range covered is 4 to 18 years. Norms for the thickness of the skin plus subcutaneous tissue, measured from anterio-posterior roentgenograms of the calf, were presented by E. L. Reynolds (*Child Development* **15,** *181 (1944)*) for ages 6.5 to 11.5 years, and by H. C. Stuart and E. H. Sobel (*J. Pediat.* **28,** *637 (1946)*) for ages from 3 months to 13 years. Normative data for Illinois Medical College freshmen (1945) have been published by T. K. Cureton ("Physical Fitness Appraisal and Guidance," *C. V. Mosby, St. Louis (1947)*), with the skinfolds measured at the cheeks, abdomen, hips, gluteals and thigh (front and rear). Beyond the college age, only data on external body dimensions

and gross weight have been available in the literature until recently.

E. L. Reynolds and T. Asakawa (*Am. J. Phys. Anthropol.* **8,** *343* (*1950*)) presented data on the thickness of subcutaneous adipose tissue (plus skin), determined from anterio-posterior roentgenograms of the leg, at the level of the greatest breadth of calf, for a sample of 100 men and 100 women. The mean ages were 37.8 and 38.0 years. The ages ranged from 19 years to 70 years, but the distribution of the ages was not specified. This information would have been highly desirable and would have served to clarify the fact that in men no correlation was obtained between age and body weight. The fact that total body weight (and fat) tends to increase with age after the cessation of height increase is well established and is incorporated in all age-height-weight tables. Only two explanations of the absence of this trend in Reynolds and Asakawa's data appear valid. Either the relationship between age and weight was curvilinear, and in that case the correlation coefficient was not the proper statistic for expression of the degree of association between the two variables, or the sample was not representative. Because the coefficient of correlation (r) appeared to indicate no relationship of breadth of fat to age in man (r = 0.008) and only moderate degree of relationship in women (r = 0.208), the norms for the breadth of fat and the other two tissue components—muscle and bone—were presented for each of the two groups as a whole. The fact that the one frequency distribution presented in full in the paper, namely the distribution of the fat/bone index, showed a marked degree of skewness (in women) suggests caution in accepting the arithmetical means and the standard deviations as the proper basis for setting up norms. The means indicated striking differences between the sexes, the men having thicker muscle and bone breadths, the women a thicker layer of subcutaneous fat. The matter of age differences in fatness, as measured by the x-ray technic, deserves a critical reexamination.

J. Brozek and A. Keys (*Brit. J. Nutrition* **5,** *194* (*1951*)) established norms for younger and older men, representative of college students (Number (N) = 133, mean age = 20.3 years) and business and professional men (N = 122, mean age = 49.0 years) on the basis of (1) specific gravity of the body, (2) thickness of five skinfolds, and (3) external dimensions (difference between the circumference of the chest and the abdomen).

The norms were derived from the frequency distributions of 8 single criteria of fatness and specify the values of the 20th, 40th, 60th, and 80th percentile. These limits divide the subjects into 5 numerically equivalent groups. The 50th percentiles (medians) as well as the means and standard deviations were also obtained. In the younger men the mean specific gravity was 1.0766, corresponding to 10.9 per cent of the body weight as fat. In the older men these values were 1.0554 and 21.3 per cent of fat.

The percentage of body fat, derived from specific gravity, appears to be the best single criterion for the evaluation of fatness in the living man. Correlation with the other criteria may be taken as an index of their validity as measures of the over-all fatness. The correlation coefficients were computed for five skinfolds and the relative body weight. The highest correlation was obtained between specific gravity and thickness of the skinfold measured on the chest above the nipple (−0.857 for younger men, −0.682 for older men).

On the basis of coefficients of correlation, means, and standard deviations, equations were developed for estimating the total fatness from single skinfolds and the relative body weight. In addition, in order to increase the precision of prediction, the six variables were combined into a single multiple-regression equation, taking into account the correlation of each predicting variable with the criterion (*i.e.*, specific

gravity), as well as the rather high intercorrelations between the predicting variables. The coefficient of multiple correlation between specific gravity and the six predictors was 0.876 for the younger men and 0.744 for the older men. The standard errors associated with specific gravity estimates were 0.0071 and 0.0085. The values of the above coefficients of correlation may be compared to the validity coefficients obtained in animal studies. E. N. Rathbun and N. Pace (*J. Biol. Chem.* **158,** *667 (1945)*), having determined the specific gravity and fat content of the eviscerated carcass in 50 guinea pigs differing widely in fatness (1.5 to 35.6 per cent of the carcass as fat), obtained a correlation coefficient of −0.972 indicating very high validity of the method.

E. Da Costa and R. Clayton (*J. Nutrition* **41,** *597 (1950)*) presented data on the specific gravity and fat content of the whole carcass of male albino rats of different nutritional states. Combining all the data (N = 99 pairs of measurements), one arrives at r = −0.73. This is a considerably lower figure than was obtained by Rathbun and Pace. There is a definite need for further investigation of the validity of estimating fat content from specific gravity, preferably using larger mammals (*e.g.*, pigs). Direct verification of the prediction formula derived for man would be desirable.

In experimental nutritional investigations in which the same group of subjects is followed over a period of time, norms of fatness are not a *necessary* prerequisite as they are for purposes of classification and nutritional surveys. But even here the norms of leanness-fatness serve in two important ways: First, they allow a proper selection and adequate characterization of the sample during the control period. Secondly, information about the magnitude of differences among individuals is helpful in establishing the biologic (as differentiated from the statistical) significance of the alterations resulting from maintenance on an experimental regimen.

Gross body weight, even when referred to the standard reference weight, remains biologically a very complex datum. For purposes of more refined description, metabolic analysis, and physiologic interpretation of weight differences, it is desirable to break down the total weight into its basic component parts: blood (plasma and cells), interstitial fluid, "active tissues" (principally muscles), and—last but not least—fat. The value of this procedure has been demonstrated in a study of prolonged caloric deficiency and subsequent refeeding (A. Keys, *et al.*, "The Biology of Human Starvation," *Univ. of Minnesota Press, Minneapolis (1950)*).

In undernutrition the body fat decreases more rapidly than muscle tissues. Conversely, in nutritional rehabilitation, especially on higher caloric intakes, the increase in body weight is accounted for largely in terms of body fat. In men who lost within six months about one-quarter of their body weight, the body fat estimated on the basis of specific gravity (Rathbun and Pace, *loc. cit.*) decreased by 70 per cent (J. Brozek, *Fed. Proc.* **5,** *13 (1946)*). Correction for excessive hydration during starvation lowers somewhat the estimated amount of body fat, with average values of 9.64 kg. during the control period and 2.78 kg. at the end of the starvation period (a decrement of 71.1 per cent). The "active tissue" was calculated as the total body weight less the sum of the fat, bone mineral, and thiocyanate space. The mean values in kilograms were 39.9 and 29.2, indicating a decrement of 26 per cent of the control value. During rehabilitation feeding the four groups of subjects who received, on the average, about 2400, 2700, 3100, and 3400 calories per day for twelve weeks, regained total body weight to the extent of 21, 30, 41, and 57 per cent of the semistarvation loss, while the recovery in body fat amounted to 17, 42, 46, and 95 per cent. In the four caloric groups deposited fat accounted for 33, 52, 51, and 67 per cent of the weight gain. This type of data con-

tributes importantly to evaluation of the efficiency of different caloric levels of diets used in nutritional rehabilitation after a prolonged period of undernutrition.

The method of estimating body fat from specific gravity represents an important advance in the quantitative morphology of the living man and should be introduced widely into nutritional studies on man in the laboratory and in the clinic. There are still several uncertainties remaining and further refinements, *e.g.*, correction for bone development, may be valuable. For the study of individuals a determination of the residual volume of air remaining in the lungs after maximal expiration is imperative. Preferably, the determination should be made simultaneously with determination of the weight of the fully submersed body. The possibility of weighing the subject submersed up to the head and estimating the head volume should be considered, especially for clinical uses. This would not eliminate the necessity for determining the residual volume. For the study of groups an average correction, established for the specific population from which the sample was drawn, is fairly satisfactory.

In field investigations, measurement of the thickness of skinfolds offers a valuable tool for characterization of the calorie nutriture of individuals and of groups of individuals. There is need for a good deal of spade work, especially at the adult level, leading to the establishment of valid population norms. However, with a machinery set up for collaborative studies, the basic data could be obtained in a short time. The standardization of measuring instruments (calipers with a known spring tension and size of contact surface) and, even more importantly, standardization of the procedures and of points of measurement, must precede any efforts designed at the establishment of usable norms for well defined sectors of the general population.

A critical evaluation and summary of the available data on measurement of fatness in man, starting with the "standard" reference weight and emphasizing the measurement of subcutaneous fat, is badly needed. The gaps existing in the available information need to be filled up by new, well coordinated research. This is essentially a task for physical anthropology, but its execution awaits stimulation and guidance by the nutritionist's needs.

FOOD FROM CULTURE OF ALGAE

Considerable interest has been shown recently in the possibility of producing food from mass cultures of microscopic plants. This has been given particular attention by those interested in the conversion of energy from sunlight to food or some other useful purpose. The increasing problem of feeding the growing population of the world may eventually bring alga culture into the forefront of research for food production. Some aspects of its possibilities are unique, and it is capable of expansion in ways different from those of conventional agriculture.

An alga commonly mentioned as a possible plant for mass culture is *Chlorella*, a green fresh-water microscopic unicellular form. For years it has been a favorite subject for research in photosynthesis and much is known of its physiology. A fundamental work on the chemical composition of *Chlorella* and the effect of environmental conditions was undertaken by H. A. Spoehr and H. W. Milner (*Plant Physiol.* **24,** *120 (1949)*). Three general processes which determine the organic composition of a plant are photosynthesis, respiration, and biosynthesis. In this study, no attempt was made to determine which process was responsible for changes observed; rather the object was to find environmental conditions which would

modify the relative proportions of carbohydrates, proteins, and lipids.

A very simple analytical means was used to determine the "degree of reduction" of the entire organic material of the plant from its elementary chemical composition. The raw materials of photosynthesis, carbon dioxide and water, have high oxygen contents. The plant reduces these compounds by elimination of oxygen. Since this is an endothermic process, the reduced compounds formed represent a proportionate storage of energy. Carbon dioxide is given a reduction level of zero. Methane, with completely reduced carbon, is assigned a reduction value, or R-value, of 100. The degree of reduction of other organic compounds can be expressed as their percentage of the degree of reduction of methane. The four principal elements of organic material, carbon, hydrogen, nitrogen, and oxygen, are determined by combustion analysis, and the R-value calculated by use of a simple formula. Variations of sulfur and phosphorus have negligible effects. The R-value is directly proportional to the heat of combustion per gram and affords an excellent but simply derived measure of energy storage in the *Chlorella* bodies produced in various experiments.

The algae were grown as pure cultures in two-liter flasks in sterilized media. After centrifuging, cells were dried and analyzed. They represented 96 to 98 per cent of the total organic matter produced by the culture. Ash content ranged from 1.5 to 20 per cent. Cells of high R-value contained less than 5 per cent ash.

The R-value varied from 38 to 63 in 328 cultures. Its change during the growth of a culture is continuous rather than stepwise. For comparison, R-values of numerous other plants were determined. Some other algae ranged from 30 to 44 in R-value; the R-value of corn leaves was 32, of sunflower leaves 38. Values for sugar maple wood are 34, for peat, 38, for soft coal, 65, and for petroleum, 81. Thus the *Chlorella* culture with highest R-value was as high as soft coal in stored energy.

Calculation of components shows that in this group of cultures the protein content varied from 58 to 9 per cent, carbohydrate from 38 to 6 per cent, and lipid from 4.5 to 85 per cent as the R-value varied from 38 to 63. Almost a linear relationship exists between increases in R-value and lipid content. Calculated values agreed closely with values from solvent extraction methods. It was shown that the R-value depends upon environmental conditions during growth rather than upon selective growth of a different strain adapted to the particular conditions.

Enrichment of the air supplied to the cultures with 5 per cent carbon dioxide increased the growth rate ten-fold. Removal of traces of oxygen from the gas mixture had no effect on growth. Numerous studies were made on mineral nutrition and favorable concentrations of various salts were found. The highest yields were obtained in total salt concentrations of 0.05 to 0.06 molal, considerably higher than frequently recommended for culture of algae. Cells producing higher R-values received 5 per cent carbon dioxide in nitrogen, under 200-watt continuous irradiation, at a temperature of 20° C.

A very striking color change, from dark-green to yellow-green and finally to yellow, accompanies increases in R-value. Both chlorophyll and carotene contents decrease. Cultures of high R-value have only 1/500 to 1/2000 of the chlorophyll content found in cultures of low R-value. The decrease of carotene content is about one tenth that of chlorophyll. This indicates a radical change in apparent efficiency of chlorophyll, a subject which should be studied further.

These studies make clear the fact that in *Chlorella* we have a plant subject to great variation in chemical composition, and that the variation can be controlled by man-made conditions. Certain conditions conducive to high R-values might well be used in the

future to supplement our supply of fatty foods. Similar survey studies on other organisms may lead to still greater variations.

If pushed to a stage of practical development, the mass culture of algae will become largely an engineering problem. They must be contained in an aqueous suspension of controlled composition, free of toxic substances, and exposed efficiently to sunlight. J. Myers, J. N. Phillips, and J. R. Graham (*Plant Physiol.* **26,** *539* (*1951*)) have made a preliminary exploration of design of a growth chamber for maximum growth rates of *Chlorella*. Growth was followed as dry weight of cells produced per unit volume of culture. Chamber thickness was varied from 6 mm. to 12 inches in diameter. When growth was plotted against time, growth was inversely related to the thickness exposed to light; growth rates became linear after about ten days, with slopes in inverse relationship to the diameter of the suspension. Carbon dioxide was not a limiting factor. The rate of light absorption determines the final linear growth rate.

An excellent means of keeping the multiplying algae supplied with adequate micronutrient elements was found in the use of a biologically inert chelating agent, ethylene diamine tetra-acetic acid. This compound forms easily reversible complexes with micronutrient elements and can maintain ionic concentrations at desirable levels throughout the life of a culture.

Full sunlight with favorable nutrient conditions produced a culture in which the algal cells occupied 25 per cent of its volume and consisted of 5 per cent of its mass. These conditions were not necessarily optimum. Limitations of growth by accumulation of toxic products, such as chlorellin, were not studied. There was no evidence of inhibiting effects due to small amounts of bacterial contamination in the cultures.

It has been forecast by several scientists that much of the world's future food supply will be grown by mass culture of some simple form of life such as *Chlorella*. These studies may be forerunners of important future developments. Problems of conversion of algae into suitable and palatable forms of food remain for the future.

METABOLIC STUDIES IN THE ADRENOGENITAL SYNDROME

Unravelling the mysteries of the relations of the anterior pituitary to the adrenals and to the sex glands has involved not only extensive experimental work but also careful clinical investigation. The latter has been substantially aided by recent availability of the pituitary adrenocorticotropic hormone (ACTH) and the adrenal cortical hormone (cortisone, Kendall's compound E). Using these two hormones as tools, recent careful metabolic studies on 3 patients with the adrenogenital syndrome have been carried out by F. C. Bartter *et al.* (*J. Clin. Invest.* **30,** *237* (*1951*)).

Three young girls (aged 10, 12 and 13) with the adrenogenital syndrome were studied. Each of the 3 illustrated most of the features of the syndrome, abnormal muscular development, deep voice, hirsutism, and clitoral enlargement. The metabolic studies included balances of nitrogen, phosphorus and calcium, serum calcium and its partition, serum glucose, serum sodium, potassium, magnesium, chlorides and carbon dioxide, and the urinary excretion of 17-ketosteroids and 11-oxysteroids. At appropriate times the patients were given injections of either ACTH or cortisone.

The results following ACTH administration were not those usually expected from the administration of this hormone. The usual negative nitrogen balance, sustained fall of circulating eosinophils and increase in urinary 11-oxysteroid excretions did not occur or were minimal. However, the urinary 17-ketosteroid excretion did rise in most

instances, even though this excretion was already high before the hormone was given. This suggested to the authors that the adrenal, which is commonly enlarged in the adrenogenital syndrome, although producing large quantities of adrenogenic material, could respond only poorly in its output of the "carbohydrate-active" substances following ACTH administration.

The administration of cortisone, however, did produce the usual negative nitrogen balance due to an increase in urinary nitrogen excretion, and, in addition, induced a striking and progressive fall in the high 17-ketosteroid excretion.

The results of the study were interpreted tentatively by the authors to indicate that the patients with the adrenogenital syndrome were unable to produce a normal quantity of carbohydrate-active hormone which led to a compensating increase in pituitary (ACTH) stimulation. Androgenic function of the adrenal, not being deficient, was thus overstimulated. The effect of cortisone, then, was to supply carbohydrate-active material which suppressed pituitary activity. Decreased ACTH production in this circumstance would secondarily decrease the androgenic activity and reduce the excretion of urinary 17-ketosteroids.

The possible effectiveness of the administration of cortisone to patients with the adrenogenital syndrome was suggested by the authors, and they are at present continuing the study with suggestive evidence of clinical improvement.

NOTES

Flavonoid Activity in Animals and Man

Interest continues in the group of compounds isolated largely from citrus fruits and originally called vitamin P by Szent-Györgi. These substances, which are now classified as flavones and include rutin, hesperidin, eriodictin, and citrin, have lost favor as vitamins, but reports continue to suggest that they may have some important pharmacologic properties.

B. Sokoloff, W. H. Eddy, and J. B. Redd (*J. Clin. Invest.* **30,** *395 (1951)*) investigated a substance which they called a complex molecule containing hesperidin-chalcone, glucose-hesperidin-chalcone, eriodictin, and quercitrin. As a preliminary test of activity, the authors observed the influence of this substance upon the rate of appearance of previously injected trypan blue into the skin of rabbits following the local application of chloroform.

Their first experiment was designed to show that a previously administered flavonoid induced inhibition of the capillary changes from the administrations of leukotaxine (Menkin). The authors do not describe the method of preparation of the leukotaxine, but did find definite inhibitions of the appearance of the previously injected trypan blue at the site of the subcutaneous leukotaxine injection.

The second series of experiments showed the protective effect of the flavonoid in tumor-bearing rats given a lethal amount of a bacterial polysaccharide (P-25, the Shear polysaccharide derived from *Serratia marcescens*). Rats bearing the August carcinoma, and averaging 8 to 9 cm. in size, were given 0.5 mg. per 100 g. of body weight of the polysaccharide. In 5 control animals, death occurred in from six and one-half to nine hours. When 3 mg. per 100 g. of body weight of flavonoid was administered a few hours prior to the polysaccharide, death was delayed, and when 10 mg. of the material was given to 12 animals death occurred in only 4 and in these was delayed from twenty-six to sixty-six hours after the polysaccharide administration.

The final experiment with rats showed a decrease in mortality and prolonged survival following total body, near-lethal x-irradiation. In the 40 control animals the mortality rate was 80 per cent. Sixty rats were given either 4 or 5 mg. of flavonoid for from ten to thirty days before irradiation. Mortality rate was reduced to 40 and finally to 10 per cent.

A clinical study of 14 patients scheduled to receive x-ray therapy for various malignant neoplasms completed the series. The flavonoid compound was administered orally in amounts from 300 to 500 mg. per day for five days prior to the initial irradiation and during the whole course of radiotherapy. The authors interpret the results as indicating, in a preliminary fashion, that the prior administration of the flavonoid compound would reduce the erythema following roentgen therapy but had no influence upon nausea and vomiting. Since no controls were provided, either using the patient as his own control or as a separate series, it is difficult to draw any final conclusions from this clinical study.

Although the clinical study does not seem to be conclusive, the animal investigations do substantiate the experimental work of others that the flavonoid compounds have a protective effect upon body capillaries.

Excretion of Choline in Liver Disease

In spite of the lack of data substantiating its effectiveness, choline and other lipotropic substances have been repeatedly recommended in the treatment of cirrhosis of the liver (*Nutrition Reviews* **8,** *238 (1950)*).

Some doubt as to the effectiveness of choline in the treatment of this and other diseases in man has been raised by the study of J. de la Huerga and H. Popper (*J. Clin. Invest.*

30, *463 (1951)*). These authors found that in normal individuals and in patients with liver and biliary tract disease essentially no choline was excreted in the urine after administration of large amounts of choline by mouth. They further observed that there was a bacterial transformation of choline in the gastrointestinal tract to trimethylamine; in fact, about two-thirds of the choline administered was excreted in the urine within twenty-four hours as trimethylamine. Whether or not the trimethylamine itself is lipotropic is not known, nor is the fate of the one-third of the choline administered which was not transformed to trimethylamine.

Eating Patterns and Problems of Children

The National Association for Mental Health, Inc., 1790 Broadway, New York 19, N. Y., has published two informative and useful booklets entitled: "Eating Problems of Children—A Guide for Doctors and Nurses," and "Eating Problems of Children —A Guide for Parents." The booklets point out that children will eat if you "let" them, do not force food, let the child's needs determine what he wants, watch when he is ready to try new things, have pleasant surroundings, are casual, friendly, patient, don't scold, nag, fuss, respect your child's likes and dislikes; children like to eat!

Recent Nutrition Books

Nutrition for Health. Holger F. Kilander. McGraw-Hill Book Company, Inc., New York, 1951. Pp. 415. Price: $3.00.

Food and You. Edmund S. Nasset. Charles C. Thomas, Springfield, Ill., 1951. Pp. 92.

Low Sodium Diet. A Manual for the Patient. Thurman B. Rice. Lea and Febiger, Philadelphia, 1951. Pp. 103.

Report of the New Guinea Nutrition Survey Expedition, 1947. Edited by E. H. Hipsley and F. W. Clements. Department of External Territories, Canberra, Australia. Pp. 308.

Perspectives in Human Malnutrition. Joseph Gillman and Theodore Gillman. Grune and Stratton, New York, 1951. Pp. 608. Price: $18.00.

Diseases in Old Age. Robert T. Monroe. Harvard University Press, Cambridge, 1951. Pp. 407. $5.00.

Handbook of Nutrition. A Symposium. Prepared under the Auspices of the Council on Food and Nutrition of the American Medical Association. 2nd edition. The Blakiston Company, Philadelphia, 1951. Pp. 717.

Vitamin Methods. Volume II. Edited by Paul György. Academic Press Inc., New York, 1951. Pp. 740.